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Daclizumab (anti-CD25) in Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a typical CD4 T cell-mediated autoimmune disease of the central nervous system (CNS) that leads to inflammation, demyelination, axonal damage, glial scarring and a broad range of neurological deficits. While disease-modifying drugs with a good safety profile and moderate efficacy have been available for 20 years now, a growing number of substances with superior therapeutic efficacy have recently been introduced or are in late stage clinical testing. Daclizumab, a humanized neutralizing monoclonal antibody against the α-chain of the Interleukin-2 receptor (IL-2Rα, CD25), which had originally been developed and approved to prevent rejection after allograft renal transplantation, belongs to the latter group.

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Apart from the promising clinical data mechanistic studies along the trials have provided interesting novel insights not only about the mechanisms of daclizumab treatment, but in general about the biology of IL-2 and IL-2 receptor interactions in the human immune system. Besides blockade of recently activated CD25+ T cells daclizumab appears to act through additional mechanisms including the expansion of immune regulatory $CD56^{bright}$ natural killer (NK) cells, the blockade of cross-presentation of IL-2 by dendritic cells (DC) to T cells, and the reduction of lymphoid tissue inducer cells.

Keywords:
Multiple sclerosis
Daclizumab
Anti-CD25
Immunomodulatory therapy
Monoclonal antibody treatment

Abbreviations:
CEL, contrast enhancing lesion
CSF, cerebrospinal fluid
DAC, daclizumab
EDSS, Expanded Disability Status Scale
MSFC, Multiple Sclerosis Functional Composite
MSIS, Multiple Sclerosis Impact Scale
NRS, Neurological Rating Scale
RRMS, relapsing-remitting multiple sclerosis
SPMS, secondary progressive multiple sclerosis

Introduction

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system (CNS). The disease is thought to be driven by autoreactive T cells that initiate the inflammatory process leading to CNS inflammation, demyelination, axonal damage and glial scarring as well as so far less well defined functional alterations (Compston and Coles 2002, Sospedra and Martin 2005). Accumulating lesions in the white and also grey matter of the CNS cause a broad range of neurological symptoms including problems of vision, sensory- or motor deficits, ataxia but also impairment of neurocognitive and autonomous functions. Early disease stages (relapsing remitting MS, RRMS) are typically characterized by complete or partial regression of relapse signs/symptoms in the following weeks. During later disease stages relapses become less frequent and eventually disappear. Further, the so-called neurodegenerative aspects of MS gain in importance and are the main reason for the steady increase of neurological disability (secondary progressive MS, SPMS). Some patients also present with primary progressive MS (PPMS). Interindividual disease heterogeneity is a hallmark of MS and renders prognostic estimates very difficult at time of diagnosis.

In spite of great efforts there is no curative treatment available for MS so far. However, the improving understanding of the pathogenesis of MS led to the development of several disease-modifying drugs in recent years. All of these target primarily the early, inflammatory disease stages of the clinically isolated syndrome (CIS) stage and RRMS. Therapeutic options in the case of acute relapse are corticosteroids (methylprednisolone) or plasmapheresis in steroid refractory relapses. Among the disease-modifying drugs are the long approved substances interferon-β (IFN-β) and glatirameracetate (GA) that are on the market since two decades (Filippini *et al.* 2013). They are characterized by moderate efficacy and a benign side effect profile. The first oral compound was fingolimod, a sphingosin-1 phosphate receptor agonist, that showed improved efficacy compared to IFN-β (Cohen *et al.* 2010). It has also been suggested to be more effective than GA, however, fingolimod and GA have so far not been directly compared, and therefore such estimates have to be made with caution. Further oral treatment options (teriflunomid, dimethylfumarate) have been introduced recently or are - depending on the country - close to licensing (Gold 2011, O'Connor *et al.* 2011, Fox *et al.* 2012, Gold *et al.* 2012). Until now natalizumab, a humanized monoclonal antibody against CD49d/very late antigen-4 (VLA-4), which is given intravenously, is suggested to be the most effective treatment (Polman *et al.* 2006). Overall natalizumab is well tolerated, but in JC polyoma virus-positive patients it can lead to subclinical and clinically apparent opportunistic virus reactivation causing progressive multifocal leukencephalopathy, an often fatal and life-threatening disease (Chen *et al.* 2009, Bloomgren *et al.* 2012). There have been 428 natalizumab-associated PML cases in MS patients until January 6, 2014 (Biogen idec, PML update online last access at March 6th 2014: http://www.biogenidec-international.com/tysabri_fact_sheet.aspx?ID=4698). An additional humanized monoclonal antibody, alemtuzumab, which is directed against CD52, a molecule of as yet unknown function on T- and other hematopoietic cells, has just been approved as well for RRMS. Alemtuzumab, like natalizumab is highly active in reducing inflammatory disease activity in RRMS (Cohen *et al.* 2012, Coles *et al.* 2012). As major side effect it causes secondary autoimmune diseases such as autoimmune thyroid disorders, idiopathic thrombocytopenic purpura and others as well as an increase

in herpes infections (Cohen *et al.* 2012, Coles *et al.* 2012). Further monoclonal antibodies are in late clinical testing including anti-CD20 mAb (ocrelizumab) and anti-CD25 mAb (daclizumab). In malignant MS forms there is still the need to apply non-specific chemotherapeutic agents like mitoxantrone or cyclophosphamide despite their well known toxic side effects and possible carcinogenicity. Mitoxantrone, which is approved for RRMS and SPMS is now used much less often due its known cardiotoxicity and the occurrence of secondary leukemia in about 0.8% or more of patients in a recent review (Marriott *et al.* 2010). Furthermore, autologous stem cell transplantation is a promising, however still experimental treatment option for patients with highly active, inflammatory MS forms if certain aspects of duration of disease, age and disability level are taken into consideration (Pfender *et al.* 2013).

Daclizumab is among the upcoming MS therapies in late stage clinical development. It is a humanized monoclonal IgG1 antibody, selectively directed against the IL-2Rα chain (CD25) leading to inhibition of assembly and signaling of the high-affinity IL-2R (Uchiyama *et al.* 1981, Uchiyama *et al.* 1981, Waldmann 2007, Martin 2012). The rationale for the development of daclizumab was the inhibition of activated T cells via blockade of IL-2 signaling for prevention of allograft rejection after kidney transplantation (Waldmann 2002). Another chimeric anti-IL-2Rα antibody is basiliximab, also used in immunosuppression protocols after kidney transplantation (Ponticelli 2013), which has, however, so far not been tested systematically in autoimmune diseases.

Increasing evidence shows efficacy of daclizumab in autoimmune diseases like MS (Bielekova *et al.* 2004, Rose *et al.* 2004, Rose *et al.* 2007, Bielekova *et al.* 2009, Rojas *et al.* 2009, Wynn *et al.* 2010, Bielekova *et al.* 2011, Gold *et al.* 2013) or noninfectious uveitis (Nussenblatt *et al.* 1999, Nussenblatt *et al.* 2003, Yeh *et al.* 2008, Wroblewski *et al.* 2011). The original rationale for investigation of daclizumab as treatment option for MS is the proposed blockade of activated, among them autoreactive, $CD25^+$ T cells, based on the concept that MS is a $CD4^+$ T cell-mediated autoimmune disease. Additional support for a role of the IL-2/IL-2 receptor (IL-2R) complex in MS stems from large genome-wide association studies, which documented that common variants (single nucleotide polymorphisms, SNPs) of IL-2 and the IL-2Rα are associated with autoimmune diseases like MS (Hafler *et al.* 2007, Lowe *et al.* 2007, Maier *et al.* 2009). Surprisingly, the blockade of activated autoreactive T cells via the high affinity IL-2R does not fully explain the efficacy of daclizumab on disease activity in MS. Other mechanisms of daclizumab like expansion and increase of immune regulatory functions of $CD56^{bright}$ NK cell functions, interference of early T cell activation via blockade of IL-2 signaling of cross presenting DCs and reduction of lymphoid tissue inducer (LTi) cells are thought to mediate the clinical effect (Wiendl and Gross 2013). Further investigations of the exact mechanisms are ongoing and will probably lead to an even better understanding of the role of IL-2/IL-2R signaling for the pathogenesis of MS.

In this review we will give a brief overview of the clinical experience with daclizumab in MS patients and it's so far identified mechanisms of action.

Clinical experience

The efficacy and tolerability of daclizumab in MS has been tested in five small, non-randomized open label studies, two randomized, double-blind phase II comparator studies and one retrospective study (table 1).

In the small open-label studies a total of 80 patients (mainly patient with RRMS, some SPMS) received daclizumab predominantly in addition to IFN-β. These proof-of-concept studies demonstrated reductions in new or total contrast-enhancing lesions on brain MRI compared with baseline, and overall patients stabilized clinically or in some cases improved (Bielekova *et al.* 2004, Rose *et al.* 2004, Rose *et al.* 2007, Bielekova *et al.* 2009, Rojas *et al.* 2009, Wynn *et al.* 2010, Bielekova *et al.* 2011, Gold *et al.* 2013). The results were promising, particularly since many of the patients had been unresponsive or responded only incompletely to prior immunomodulatory treatments. Furthermore, while the primary outcomes, the reduction of contrast-enhancing MRI activity had been met, patient numbers were too small to establish efficacy with respect to clinical outcomes.

The first randomized, double-blind, placebo-controlled phase II trial (CHOICE) compared efficacy and safety of subcutaneous high dose (2mg/kg every 2 weeks) with low dose (1mg/kg every 4 weeks) daclizumab as add-on therapy to IFN-β in 230 RRMS patients over a period of 6 month (Wynn *et al.* 2010). The number of new or enlarged gadolinium (Gd) enhancing lesions on brain MRI as primary endpoint was reduced by 72% ($p<0.01$) in the DAC high-dose group and by 25% ($p=0.51$) in the DAC low-dose group compared to IFN-β-treated patients. The clinical parameters (relapse rate, disability progression) as secondary and tertiary endpoints were not changed. High-dose DAC add-on therapy to IFN-β was herewith shown to be more effective than IFN-β alone or in combination with low-dose DAC treatment, but the study duration of 6 months may have been too short to demonstrate an effect on clinical outcomes. DAC saturation of the IL-2Rα in peripheral blood CD4 T cells was complete in the DAC high-dose and low-dose treatment groups (Wynn *et al.* 2010). This finding indicates either an effect of higher DAC dose on IL-2Rα on tissue cells, or off target effects that lead to better efficacy of higher doses. The absolute numbers of B- and T cells were not changed in the periphery, but $CD25^+$ T cells dropped by approximately 25%. Furthermore, consistent with the findings in the above pilot trials (Bielekova *et al.* 2004, Bielekova *et al.* 2011), the numbers of $CD56^{bright}$ NK cells was 7-8 times higher in DAC treated patients compared to placebo treated patients, and these effects reverted after discontinuation of DAC (Wynn *et al.* 2010).

A recent study used daclizumab high-yield process (DAC HYP), a new formulation of daclizumab with different glycosylation pattern, which had been designed for reduced antibody-dependant cellular cytotoxicity (Gold *et al.* 2013). Two doses of DAC HYP (150mg and 300mg every 4 weeks s.c.) monotherapy were examined in a randomized, placebo-controlled phase II trial (SELECT) compared to placebo in RRMS patients (n=621) over 52 weeks (Gold *et al.* 2013). The annualized relapse rate served as primary endpoint and was 0.46 in the placebo group, 0.21 in DAC HYP 150mg and 0.23 in DAC HYP 300mg group, corresponding with a relapse rate reduction of 54%/50% respectively. After one year of DAC HYP treatment 39% of patients was free of disease activity (no relapses, no disease progression, no new or enlarged T2 lesions, no new Gd-enhancing lesions) compared to 11% of

patients received placebo. Reduction of disability progression by EDSS score could also be demonstrated in the DAC HYP-treated groups: the placebo group showed an estimated (Kaplan-Meier) disability progression of 13% (n=25), DAC HYP 150mg group showed 6% (n=11) and DAC HYP 300mg group showed a disability progression of 8% (n=15). EDSS score after 52 weeks compared to baseline was 0.09 in placebo group, -0.08 in DAC HYP 150mg group and 0.05 in DAC HYP 300mg group. Improvement in the MSIS-29 physical score was noted in the DAC HYP 150mg but not in the DAC HYP 300mg score compared to placebo (Gold *et al.* 2013).

The results of the MRI endpoints were in line with the reduction of clinical disease activity. Reduction of new or enlarged T2 lesions was 70% in the DAC HYP 150mg and 79% in the DAC HYP 300mg group compared to placebo groups after 52 weeks (Gold *et al.* 2013).

Table 1: Clinical trials of daclizumab in multiple sclerosis

Study name, type and reference	MS course, number of individuals	Treatment arms	Duration	Clinical outcome	Radiological outcome
open label phase II (Rose *et al.* 2004)	RRMS/ SPMS n=19	DAC (n=16), DAC + IFN-β/ corticosteroids (n=3)	5-25 months	↓ EDSS score	ND
open label phase II (Bielekova *et al.* 2004)	RRMS/ SPMS n=11	DAC + IFN-β	30 weeks	↓ relapse rate ↑ Scripps NRS ↔ EDSS	↓ 78% new CELs ↓ 70% total CELs expansion of $CD56^{bright}$ NK cells
open label, phase II (Rose *et al.* 2007)	RRMS (failing IFN-β) n=9	DAC + IFN- β, then DAC alone	up to 27.5 months	↓ relapse rate ↑ Scripps NRS ↓ EDSS	↓ >75 % new CELs ↓ >75 % total CELs
open label phase II (Bielekova *et al.* 2009)	RRMS/ SPMS (failing IFN-β) n=15	DAC + IFN-β, then DAC alone	5.5 month combination, 10 month DAC monotherapy	↑ MSFC ↑ Scripps NRS ↓ EDSS	↓ 72% new CELs
open label phase II (Bielekova *et al.* 2011)	RRMS with high inflammatory activity n=16	DAC	54 weeks	↑ MSFC ↑ Scripps NRS ↓ EDSS	↓ 88% new CELs expansion of CD 56^{bright} NK cells in peripheral blood and CSF
retrospective study (Rojas *et al.* 2009)	RRMS n=12	DAC	24-60 months	↓ relapse rate ↓ EDSS	ND
CHOICE study (randomized placebo controlled phase II trial) (Wynn *et al.* 2010)	RRMS/ SPMS n=230	IFN-β + placebo (n=77) IFN-β + low dose DAC (n=78) IF-β + high dose DAC (n=75)	24 weeks	reference group ↔ relapse rate ↔ MSFC3 ↔ EDSS ↔ relapse rate ↔ MSFC3 ↔ EDSS	reference group ↓ 25% new/ enlarged CELs ↓ 72% new/ enlarged CELs
SELECT study (randomized placebo controlled phase II trial) (Gold *et al.* 2013)	RRMS n=600	placebo low dose DAC-HYP high dose DAC-HYP	52 weeks	reference group ↓ relapse rate ↓disability progression ↓ MSIS-29 physical score ↓ relapse rate ↓ disability progression ↔ MSIS-29 physical score	reference group ↓ 70% new/ enlarged CELs ↓ 79% new/ enlarged CELs

Abbreviations: CEL, contrast enhancing lesion; CSF, cerebrospinal fluid; DAC, daclizumab, EDSS, Expanded Disability Status Scale; HYP, high yield process; MSFC, Multiple Sclerosis Functional Composite; MSIS, Multiple Sclerosis Impact Scale; ND, not determined; NRS, Neurological Rating Scale; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis;
Adapted from (Martin 2012, Wiendl and Gross 2013)

Safety and tolerability

The clinical experience with daclizumab in MS patients shows overall a good safety profile so far (Bielekova *et al.* 2004, Rose *et al.* 2004, Rose *et al.* 2007, Bielekova *et al.* 2009, Rojas *et al.* 2009, Wynn *et al.* 2010, Bielekova *et al.* 2011, Gold *et al.* 2013). Most common adverse events were transient elevation of liver enzymes, infections and cutaneous adverse events (generally mild to moderate, local or generalized rashes that responded well to low dose corticosteroids). In the SELECT trial 5 serious cutaneous adverse events were reported (one case of each: rash, atopic dermatitis, allergic dermatitis, exfoliative dermatitis, and erythema nodosum) compared to none on the placebo groups (Gold *et al.* 2013). Typical adverse events of other injection therapies in MS such as flu-like symptoms or injection-side reactions have not been documented.

The rates of adverse events and serious adverse events in the largest trials (CHOICE and SELECT) were comparable to patients treated with placebo (Wynn *et al.* 2010, Gold *et al.* 2013). The rate of serious infections was slightly increased in the DAC treatment groups, but no specific pattern of organ system involvement was seen and no opportunistic infections have been documented (Wynn *et al.* 2010, Gold *et al.* 2013). No cases of PML were seen in MS patients treated with DAC. Herpes virus infections as typical complications of impaired T cell immunity were similarly distributed between placebo and DAC groups in the SELECT trial (Gold *et al.* 2013). One death of a patient receiving DAC HYP was reported in the SELECT trial, where a contribution of DAC HYP could not be excluded. The patient recovered from a serious rash and died of local complications of a psoas abscess that led to mesenteric thrombosis and ischemic colitis (Gold *et al.* 2013).

Apart from MS there is long-standing prior clinical experience with daclizumab as a treatment to prevent the rejection of allografts. A recent Cochrane analysis showed a good safety profile of DAC in 10.520 patients, who received either DAC, placebo and in most cases DAC with other immunosuppressive drugs (Webster *et al.* 2010).

Taken together for MS patients longer term experience in larger patient cohorts are needed to evaluate the risk for rare adverse events like malignancies or infections. The results of the ongoing phase III trial (DECIDE) and the long term follow up at 8 years of treatment in patients of the SELECT trial will provide further information in the next years. Based on the experience in MS until now DAC can be considered as a well tolerated therapy option in MS that is safe in the vast majority of patients.

Mechanistic insight

The high affinity IL-2 receptor (IL-2R) is a heterotrimer consisting of the IL-2R alpha chain (IL-2Rα, CD25) and the IL-2R beta- and gamma chains (IL-2Rβ and IL-2Rγ) (Malek 2008). It is only expressed on activated T lymphocytes and serves as their major growth factor receptor by binding IL-2. The IL-2Rα is private, i.e. only expressed in the IL-2R, and does not contain an intracellular signaling domain, and binding to IL-2Rα alone does not result in T cell activation (Malek 2008). The high affinity IL-2R heterotrimer is expressed on recently activated T cells and regulatory T cells (Tregs) (Malek 2008).

However, an IL-2R heterodimer also exists and is expressed on different cell types consisting of the IL-2R beta chain (IL-2Rβ, CD122), which is shared with the IL-15 receptor, and the so-called common gamma-chain of cytokine receptors (IL-2Rγ, CD132), which is also found in receptors for IL-4, IL-7, IL-9, IL-15, and IL-21. The latter heterodimer is constitutively expressed on the surface of NK cells. Both IL-2Rβ and IL-2Rγ contain intracellular signaling domains, and binding of IL-2 to the intermediate affinity IL-2R leads to activation of NK cells, which then use IL-2 also as their growth-promoting factor. Daclizumab blocks the high affinity interaction of IL-2 and its receptor by binding specifically to the TAC epitope/binding site on the IL-2Rα chain (Waldmann 2007, Malek 2008). The rationale to use DAC in the context of MS was based on its known effects with respect to blocking the expansion of recently activated T cells, for which it serves as essential mediator of T cell growth and survival (Waldmann 2002) and the assumption that this may be useful in MS as a CD4 T cell-mediated autoimmune disease (Sospedra and Martin 2005, McFarland and Martin 2007). Interestingly, the exploration of manipulating IL-2 or the IL-2R components in animal models indicated that blocking IL-2R in humans could be dangerous. Genetic knock-out (ko) of IL-2 and of one of the three components of the IL-2R universally resulted in severe pathologies, i.e. inflammatory bowel disease in IL-2 ko mice (Sadlack *et al.* 1993, Sadlack *et al.* 1995), lymphoproliferative diseases and autoimmunity in IL2-Rα ko mice (Willerford *et al.* 1995), lethal autoimmunity in IL2-Rβ ko mice (Suzuki *et al.* 1995) and severe combined immunodeficiency in IL-2Rγ ko mice (Leonard *et al.* 1995). When a blocking anti-IL2-Rα antibody was used in a rat model of experimental autoimmune encephalomyelitis (EAE), the animal model of MS, no effects have been observed (Engelhardt *et al.* 1989). The combined experience from these animal studies indicated that it would be either ineffective or too dangerous to block the IL-2Rα in humans. While a detailed comparison of the exact consequences of IL-2Rα blockade or ko of various IL-2R components between humans and mice has not been performed, it suggests, however, that there are profound differences between the rodent and human immune system and that certain functions of IL-2/IL-2R interactions, e.g. its role for Tregs, are less critical in humans than in mice.

When daclizumab was tested, based on the above assumptions and prior knowledge, in autoimmune diseases, i.e. first in treatment-resistant uveitis (Nussenblatt *et al.* 1999, Nussenblatt *et al.* 2003) and then in MS (Bielekova *et al.* 2004, Rose *et al.* 2004), but also in HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Lehky *et al.* 1998) it was surprising that blocking the high affinity IL-2R by masking its IL2-Rα component resulted in only modest declines of T cell numbers. Further, this reduction was much smaller than expected when considering the profound reduction of Gd contrast-enhancing MRI lesions (Bielekova *et al.* 2004). Detailed *ex vivo* immune studies in daclizumab treated MS patients then revealed among other modest alterations a previously unknown effect, i.e. a highly significant increase of a subpopulation of NK cells that expresses CD56 (NCAM) at high levels ($CD56^{bright}$ NK cells) (Bielekova *et al.* 2006). This effect has since then been confirmed in several other clinical trials with daclizumab in MS and uveitis (Li *et al.* 2005, Bielekova *et al.* 2009, Wynn *et al.* 2010, Bielekova *et al.* 2011, Sheridan *et al.* 2011, Gold *et al.* 2013).

Effect of Daclizumab on NK cells

As mentioned above daclizumab treatment leads to 7-8 fold increased CD56^{bright} NK cell numbers in the peripheral blood and the CSF (Bielekova *et al.* 2009, Wynn *et al.* 2010, Bielekova *et al.* 2011, Sheridan *et al.* 2011, Gold *et al.* 2013). Furthermore increased numbers of CD56^{bright} NK cells correlated positively with clinical response (Bielekova *et al.* 2006, Wynn *et al.* 2010). In the recent SELECT study patients with highest CD56^{bright} NK cell counts showed a marked reduction in T2 lesions on brain MRI compared to placebo treated patients and DAC-treated patients with low CD56^{bright} NK cell counts (Gold *et al.* 2013). Interestingly, pregnancy related relapse rate reduction in the 3rd trimester is also associated to increased CD56^{bright} NK cell counts (Airas *et al.* 2008).

So far no direct interaction of daclizumab with NK cells is known. The proposed mechanism is thought to be mediated via increased local IL-2 availability for the intermediate IL-2R on NK cells and therefore increased IL-2 signaling. Blockade of the high-affinity IL-2R leads to increased IL-2 production of these cells (Malek 2008) and reduced IL-2 consumption (Bielekova *et al.* 2006, Martin *et al.* 2010) at the same time, both resulting in increased availability of IL-2 for NK cells. Notably, the intermediate-affinity IL-2R is higher expressed on CD56^{bright} NK cells (also known as regulatory NK cells) compared to T cells or CD56^{dim} NK cells (Bielekova *et al.* 2006). Furthermore CD56^{bright} NK cells isolated from DAC-treated patients showed increased immune regulatory function such as enhanced cytotoxicity against recently activated autologous CD4^{+} T cells (Bielekova *et al.* 2006). One possible mechanism of CD56^{bright} NK cell mediated killing of T cells could act via release of the serine protease granzyme K that is expressed in granules of CD56^{bright} NK cells and induces caspase independent apoptosis (Bratke *et al.* 2005, Zhao *et al.* 2007, Jiang *et al.* 2011).

Recent studies showed also the ability of CD56^{bright} NK cells to kill other immune cells like immature DCs, immature microglia and activated macrophages (Cooper *et al.* 2001, Cooper *et al.* 2001, Della Chiesa *et al.* 2003, Lunemann *et al.* 2008).

The interesting data that emerged in the context of the use of daclizumab indicates that the role of NK cells in immune regulation in the context of autoimmune diseases like MS should be investigated further in the future.

Effects of daclizumab on other immune cells

The fact that not all patients, who responded clinically to daclizumab treatment, show increased CD56^{bright} NK cell counts raised the questions whether other mechanisms are involved (Wuest *et al.* 2011). During early steps of immune activation DCs are able to secrete IL-2 and express the IL-2Rα chain earlier than T cells. To activate resting T cells DCs can form immune synapses, secrete IL-2 into the synaptic cleft and express IL-2Rα chain at this site. Together with IL-2R β and γ chains from the T cell side the high affinity IL-2R heterotrimer can be formed. This mechanism is referred to as trans-presentation (Schluns 2011, Wuest *et al.* 2011). A first study suggests a role for daclizumab in blocking trans-presentation of IL-2 from DCs to T cells (see figure 1) (Wuest *et al.* 2011).

Effects of daclizumab on CD4^{+} T cells

IL-2 drives immune regulation via stimulation of Treg cells as well as inflammation via stimulation of effector T cells. Mice lacking IL-2, IL-2Rα or IL-2Rβ suffer from lethal autoimmunity that can be cured with wild type $CD4^+CD25^+$ Treg cells indicating a significant role of Treg cells on immune homeostasis *in vivo* (Malek 2008, Liao *et al.* 2013).

The so far largest and most recent clinical study (SELECT) investigated the effect of DAC HYP on Treg numbers in 370 patients over a period of 52 weeks from baseline. Treatment of daclizumab caused a reduction of nearly 40% of $CD4^+CD127^{low}Foxp3^+$ Treg cells established after 8 weeks of treatment lasting over 52 weeks of treatment (Gold *et al.* 2013). Interestingly Treg numbers were not predictive for MS outcomes or adverse events in the SELECT study (Gold *et al.* 2013). Other studies showed additionally an impaired immunosuppressive function in the quantitatively reduced Treg compartment without effect on brain inflammation (Oh *et al.* 2009, Martin *et al.* 2010, Martin 2012). Despite the dependency of Treg cells on IL-2, a low level of IL-2 signaling seem to be sufficient to maintain immune homeostasis (Yu *et al.* 2009). Furthermore, signaling through other cytokines may compensate a lack of IL-2. In line with this hypothesis increased expression of IL-7Rα (CD127) on Treg has been shown after daclizumab treatment (Martin *et al.* 2010), although the role of IL-7 signaling for Treg function is still controversial (Heninger *et al.* 2012). Taken together, the clinical relevance of reduced Treg numbers after dacluzimab treatment is not fully understood and other immune regulatory mechanisms, e.g. increase of immune regulatory $CD56^{bright}$ NK cells, may compensate decreased Treg function.

Direct effects of reduced IL-2 signaling by daclizumab treatment on other T cell subsets, especially $CD4^+$Th2 cells and $CD8^+$ effector and memory cytolytic T cells, were expected from *in vitro* data (Liao *et al.* 2013). *In vivo* only slight changes in numbers of $CD4^+$ and $CD8^+$ T cells, proliferation and cytokine production were seen (Bielekova *et al.* 2006, Bielekova *et al.* 2009).

Effect of daclizumab on lymphoid tissue inducer cells

Lymphoid tissue inducer (LTi) cells belong to the group of innate lymphoid cells (ILC) that are suggested to play an important role in autoimmune diseases (Sanos and Diefenbach 2013). In MS increased numbers of circulating LTi cells have been observed (Perry *et al.* 2012). Pathogenetically LTi cells could play an important role by the generation of ectopic meningeal lymphoid follicles that lead to meningeal inflammation (Lucchinetti *et al.* 2011, Spits and Di Santo 2011). Interestingly daclizumab treated MS patients show a reduction in LTi cells that correlates with an increase of $CD56^{bright}$ NK cells (Perry *et al.* 2012). As $CD56^{bright}$ NK cells and LTi cells originate from the same IL-2Rβ positive ILC the balance between these cells seem to be shifted by IL-2/IL-15 signaling via the intermediate IL-2R towards $CD56^{bright}$ NK cells (Freud *et al.* 2005, Perry *et al.* 2012).

Inhibition of IL-2 signaling as therapeutic option

Beside daclizumab two other molecules modulating IL-2 signaling are in clinical testing. Ruxolitinib and Tofacitinib are both JAK (Janus kinase) inhibitors that act downstream of the IL-2R but also of other

cytokine receptors (Liao *et al.* 2013). Especially inhibition of JAK3 is expected to have immune regulatory effects. As IL-2 is a pleiotropic cytokine inducing immunoregulatory as well as pro-inflammatory pathways further studies are mandatory to characterize the exact mechanisms.

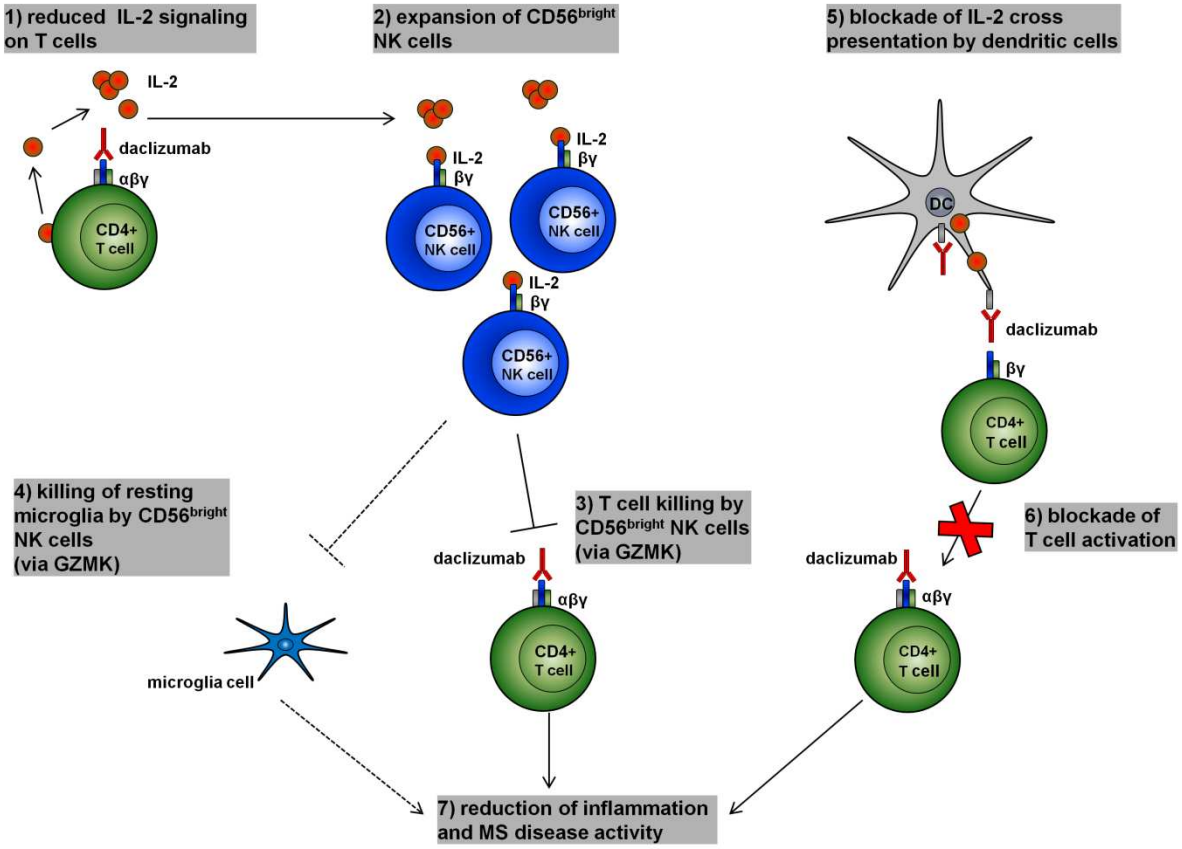

Figure 1: Proposed mechanisms of daclizumab in MS

1) The autocrine IL-2 stimulation is blocked by daclizumab in activated $CD25^{+}$ T cells. 2) Therefore more IL-2 is available for cells expressing the intermediate affinity IL-2R, e.g. $CD56^{bright}$ NK cells resulting in expansion of these cells. 3) $CD56^{bright}$ NK cells have immunoregulatory properties like granzyme k (GZMK)-mediated killing of activated, possibly autoreactive T cells and probably also killing of microglia cells (4, only shown *in vitro* so far). 6) Blockade of IL-2Rα on DC prevents cross presentation of IL-2 to T cells presenting the intermediate IL-2R. 6) Therefore further activation of these T cells is diminished by daclizumab. 7) All of these pathways result (indirect via $CD56^{bright}$ NK cells or DC) in the prevention of activation of autoreactive T cells in MS patients leading to decreased disease activity. Solid arrows show well established mechanisms, dashed arrows show possible modes of action. Adapted from (Wiendl and Gross 2013).

Outlook

Among the upcoming drugs for MS, daclizumab represents an interesting and promising addition even though a number of anti-inflammatory/immunomodulatory compounds are already available. One advantage is its monthly subcutaneous administration, which is convenient for patients and assures patient adherence. More importantly, when considering its efficacy, daclizumab is expected to be similar to natalizumab and therefore slightly more active than the best oral compounds, although such estimates are difficult without direct comparison of the drugs. With respect to side effect profile, daclizumab at least at the moment appears superior to both natalizumab and alemtuzumab, because it neither carries the risk for PML nor for the development of secondary autoimmune diseases. Skin reactions and transient elevations of liver enzymes were usually observed at the onset of treatment and in most instances not of concern. Also, the control of latent/persistent infections with herpes viruses and JCV does not appear to be compromised, and the same holds true for new infections with viruses or bacteria. One of the most interesting aspects of the development of daclizumab has been the identification of novel mechanisms of action including the expansion of $CD56^{bright}$ NK cells, the inhibition of IL-2 trans-presentation by recently activated DCs and the effects on lymphoid tissue inducer cells. Particularly the effects on NK cells, which correlate well with the inhibition of inflammatory CNS activity and clinical outcomes, represent an important and previously unrecognized mechanism of action of blocking CD25. Further, measuring the increase of $CD56^{bright}$ NK cells during daclizumab treatment can be used as a relatively easy to detect biomarker for responsiveness. The studies on mechanistic aspects of daclizumab along the clinical development highlight the importance of such studies not only for gaining a better understanding of the mechanism of action of the drug, but also about basic immunological interactions related to IL-2/IL-2R signaling and functions.

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